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Development and Clinical Evaluation of Immunoluminometric Assays for Lactoferrin and Elastase-α_1-Proteinase Inhibitor Complexes in Body Fluids with Special References to Bronchoalveolar Lavage and Neonatal Sepsis

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Dedicated to Professor H. G. Hansen on the occasion of his 65th birthday

Summary: Immunoluminometric assays for lactoferrin and elastase-α_1-proteinase inhibitor complexes were developed using solid-phase methodology, which has already been published from this laboratory. The aim of the study was to develop a rapid method to see whether elevated granulocyte activity was present in the lung, as for example in neonatal sepsis. The lactoferrin assay gave reliable results within 30 minutes, the elastase-α_1-proteinase inhibitor complexes, within 5 hours.

The correlation between both analytes was good, so that the lactoferrin assay could replace the elastase-α_1-proteinase inhibitor assay in emergency cases. The lactoferrin assay was used for rapid answer, the elastase-α_1-proteinase inhibitor complex assay for "fine" monitoring of the progress of the disease. Both assays could be used to measure concentrations in plasma or bronchoalveolar lavage using a 10 µl sample. Plasma for the elastase-α_1-proteinase inhibitor complex determination had to be diluted 1 : 50 before being assayed.

Only EDTA plasma was used in the assay, as either heparin plasma or serum resulted in granulocyte destruction, thus giving rise to elevated, and non-reproducible results.

The results from bronchoalveolar lavage show an excellent correlation between elastase-α_1-proteinase inhibitor complexes and lactoferrin.

No interference was seen from lipaemic or icteric plasma samples. Results from haemolytic samples i. e. where lysis of erythrocytes and leukocytes had occurred, had to be treated with care if no clinical indication of intravascular haemorrhage was present. The assays lend themselves to perinatal diagnosis, as the total volume

of plasma or lavage needed is theoretically under 50 µl, i.e. ethically acceptable for regular monitoring of neonates. As far as could be determined, both assays were specific and robust.

The elastase-α_1-proteinase inhibitor immunoluminometric assay correlated well with the commercial ELISA test kit in EDTA plasma (ELISA = (0.93 × ILMA) − 41, n = 52, r = 0.93) showing that both assays measured similar complexes.

Introduction

It is often difficult to determine causes of lung dysfunction and sepsis in neonates. The aim of this study was to combine the sensitivity of luminescent detection systems and the specificity of leukocyte analytes (in this case, elastase and lactoferrin) for use in a paediatric clinic and intensive care unit.

We describe the development and clinical evaluation of immunoluminometric assays for lactoferrin and elastase-α_1-proteinase inhibitor complexes in plasma and bronchoalveolar lavage samples.

Both analytes monitor granulocyte activity (1−3), and are useful in monitoring inflammatory disease with possible bacterial involvement, where other analytes, for example C-reactive protein alone, provide insufficient data. In this article special mention is made of lung diseases, especially pneumonia and neonatal sepsis. Since the assay is very sensitive, requiring a sample volume of less than 50 µl for the determination of both components, both tests are suitable for perinatal diagnosis and monitoring.

The elastase is present in the primary vesicles of polymorphonuclear leukocytes (4) and is set free during normal or "frustrated" phagocytosis or cell destruction. Lactoferrin is not only present in polymorphonuclear leukocytes (5), but is also present in pancreatic juice (6), milk (7) and synovial fluid (5), so that the determination in plasma is not as specific as that of elastase-α_1-proteinase inhibitor complexes. Theoretically all body fluids are suitable for the determination of elastase complexes and lactoferrin, dependent upon the site of inflammation. Bronchoalveolar lavage may form the ideal sample if granulocyte activity is to be monitored in respiratory disease.

EDTA plasma was the material of choice for blood concentration measurements (8). Although in practice citrate and oxalate plasma could be used, the dilution factor (1 part citrate/oxalate + 9 parts blood) must be taken into consideration. A commercial enzyme immunoassay (PMN-Elastase ELISA, Merck, Darmstadt, D), which requires regular routine experience to obtain optimal results, is available for measuring elastase-α_1-proteinase inhibitor complexes. The results for the Merck test are available about 6 hours after taking the sample.

A solid-phase radioimmunoassay for lactoferrin has been published by *Bennett & Mohle* (9), in which serum concentrations were higher than plasma concentrations, and in which the "leak" process was followed in serum, heparin and EDTA plasma over several hours.

The assays developed and validated in the present work are suitably robust and reproducible for routine use, and do not involve the use of a radioactive label.

Materials and Methods

Immunoluminometric assays using polystyrene balls as solid-phase have been published previously from this laboratory (10−14).

Antisera

Antiserum to α_1-proteinase inhibitor was purchased from Cappel Laboratories (Organon Teknika N.V., Turnhout, B).

Antiserum to human elastase was obtained from Serotec (Camon GmbH, Wiesbaden, D).

The two antisera for the lactoferrin assay were obtained from Cappel and Dakopatts (Hamburg, D) respectively.

Polystyrene balls (6.4 mm diameter) were bought from Spherotech Kugeln (Fulda, D).

Antibodies were labelled with N-(7-(4-aminobutyl-N-ethyl) naphthalene-1,2-dicarboxylic acid hydrazide) hemisuccinamide (ABEN-H) as previously published (10).

Standards

Lactoferrin and α_1-antitrypsin (α_1-proteinase inhibitor) were obtained from Sigma, Deisenhofen, D, elastase from Protogen, Läufelfingen, CH.

The elastase standard was complexed with an excess of purified α_1-proteinase inhibitor in a molar ratio of 1:35 (i.e. under physiological conditions). Complex formation was monitored by loss of elastase activity, determined photometrically with the synthetic substrate Me-O-Suc-$(Ala)_2$-Pro-Val-nitroanilide (Bachem, Bubendorf, CH). The nominal values of the elastase-α_1-proteinase inhibitor are given in terms of the elastase content.

Lactoferrin standards were made up in foetal calf serum as this was shown to be free of immunoreactive lactoferrin.

Samples

EDTA blood samples were obtained from patients with and without lung infections, as well as from healthy blood donors with normal blood sedimentation rate, normal blood smear, aminotransferases and γ-glutamyl transferase. The blood samples were left to stand for 30 minutes at 4 °C before being centrifuged at 2000 *g* for 10 min. The supernatant was then

removed, portioned and stored at −20 °C until assayed. Full EDTA blood samples older than 2 hours could not be used as the "leak process" had already started, giving rise to falsely elevated results.

Bronchoalveolar lavage was obtained from different patient groups, using a published standardised method (15).

Tables 1a and 1b show the assay schemes for the elastase-α_1-proteinase inhibitor complex assay (PMN-Elastase) from Merck, (Darmstadt, D) and the immunoluminometric assay developed in this laboratory.

Table 1c shows the assay flow-diagram for the lactoferrin immunoluminometric assay.

Tab. 1a. Flow diagram of the Merck PMN-Elastase-Enzyme-Immunoassay

1000 µl wash solution.
1 coated tube.
5–20 minutes incubation at 20–25 °C.

Aspirate.

500 µl sample (1 : 50 dilution).
500 µl dilution buffer.

1 h incubation at 20–25 °C, then
wash with 3 × 1 ml wash solution.

500 µl antibody-enzyme solution (alkaline phosphatase).
1 h incubation at 20–25 °C.

2 ml wash solution.
Stand for 10 min and aspirate.

500 µl substrate solution.

1.5 h incubation at 20–25 °C.
Add 500 µl stop solution (2 mol/l NaOH)
and measure absorbance at 405 nm

Tab. 1b. Flow diagram of the elastase-α_1-proteinase inhibitor complex Immunoluminometric Assay (ILMA)

200 µl sample/standard (plasma 1 = 50 dil., lavage 1 : 1 – 1 : 25 dil.)
1 anti human PMN-elastase-coated ball.
3 h incubation at room temperature on horizontal rotator (170 min^{-1}).
Wash with 2 × 5 ml 0.25 ml/l Tween 20.

200 µl ABEI-labelled anti-α_1-proteinase inhibitor in assay buffer*).
2 h incubation (process as above).
Transfer ball to measuring cuvette,
add 300 µl catalyst solution,
load luminometer (LB 950 or LB 952) and initiate light reaction with 300 µl alkaline peroxide.
Integrate light signal over 10 or 2 s respectively.

Tab. 1c. Flow diagram of the Lactoferrin ILMA

10 µl sample/standard.
200 µl Anti-Lactoferrin-ABEN (Dako) in assay buffer*).
1 Anti-Lactoferrin-coated ball (Cappel).

Incubate 30 min on horizontal rotator (170 min^{-1}) and process as in table 1b

*) assay buffer:
0.025 mol/l phosphate, 0.15 mol/l sodium chloride, 0.25 ml/l Tween 20, pH 7.6.

Instruments

All measurements were performed on either an LB 950 or LB 952 automated luminometer (Laboratorium Prof. Dr. Berthold, Wildbad, D) using a 2 s integral (LB 952) or a 10 s integral (LB 950) time. Both luminometers had a capacity of 250 samples.

Horizontal rotators (Heidolph Horizontalrotierer, Abbott, Wiesbaden, D) were used for the incubation steps. The washing procedures were performed using an automatic washing system (Pentawash or Pro-quantum, Abbott, Wiesbaden, D).

Results

Table 2 shows the imprecision using compound precision profiles and inter-assay imprecision for both assays.

Figure 1 shows typical standard curves for elastase-α_1-proteinase inhibitor and lactoferrin assays, respectively.

The effect of 5 different dilution media for the elastase-α_1-proteinase inhibitor assay was tested, the results being shown in table 3.

Recovery experiments were carried out on two plasma samples with low and high concentrations, the results being listed in table 4. The elastase-α_1- proteinase inhibitor recovery was determined additively, the lactoferrin recovery by using an admixture of 2 samples. In addition the mean recovery of elastase from the addition of two plasma samples was measured in 8 consecutive assays: Plasma 1 – 98 µg/l, Plasma 2 – 347 µg/l. Expected value – 445 µg/l, mean value found – 472 µg/l, mean recovery 106%.

Tab. 2. Imprecision data for elastase-α_1-proteinase inhibitor complex and lactoferrin assays (mean of duplicate values used for calculation)

a) *Precision profiles*

1) Elastase complex (24 assays)
50–500 µg/l n = 319 CV = 5.17%
> 600 µg/l n = 130 CV = 4.72%

2) Lactoferrin (22 assays)
0.1–1 mg/l n = 469 CV = 5.80%
> 1 mg/l n = 41 CV = 4.92%

b) *Interassay variation*
(derived from means of duplicate determinations)

1) Elastase complex (µg/l, n = 38 assays)

		mean	CV %
Control plasma	1	85	11.3
	2	141	7.7
	3	541	12.9

2) Lactoferrin (mg/l, n = 40 assays)

		mean	CV %
Control plasma	40	0.97	11.8
	41	0.32	6.9
	35	0.59	6.1

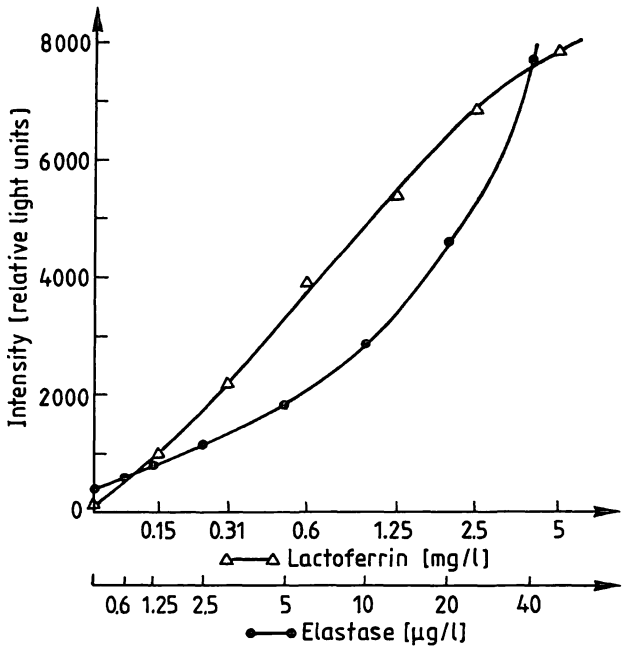

Fig. 1. Standard curves of both assays, performed as described in tables 1b + 1c.
The units on the ordinate are relative light units and are directly proportional to the number of photons emitted. The standard concentrations can be seen on the abscissa.

The mean lower detection limit of the elastase-α_1-proteinase inhibitor complex assay, measured as µg/l elastase was 0.3 µg/l (12 fg/tube) (or in the plasma assay 15 µg/l — allowing for the 50-fold sample dilution).

This represented the mean + 3 s value of the zero standard read from 20 standard curves. This value did not overlap the −3 s value of the first standard point. For lactoferrin in a similar study, the mean lowest detection limit was 0.04 mg/l (0.4 pg/tube) using a 10 µl plasma sample.

Lipaemic and icteric sera did not interfere in the assay, probably due, amongst other things, to the small sample volume (less than 4% of the total incubation volume).

Haemolysed samples were discarded unless intravascular haemolysis was expected or was not to be excluded (e. g. Rh-erythroblastosis). These findings comply with those of *Neumeier* et al. (17). Purified haemoglobin did not affect the results of other assays when added to the plasma sample to be measured at a concentration of 1 g/l.

The specificity of the elastase assay was tested with pure human α_1-proteinase inhibitor (Sigma) complexed with human pancreatic elastase and porcine pancreatic elastase. Neither preparation reacted in the assay even at concentrations of 20 mg/l elastase complexes.

Tab. 3. Effect of dilution media on the shape of the standard curve and the results read from the relevant curve for the elastase-α_1-proteinase inhibitor assay

Elastase standard (µg/l)	Buffer 1	Buffer 2	Buffer 3	Buffer 4	Buffer 5
0	600	500	460	520	460
9	800	700	620	850	700
18	1100	950	950	1250	1150
36	2000	1550	1550	1800	1720
72	4910	2650	2850	2740	2660
90	6010	3390	3310	3610	3360
180	7890	6190	5120	6210	5070
Sample No	Elastase µg/l				
1	45	55	40	42	45
2	37	52	37	39	42
3	60	66	59	60	59
4	43	54	40	44	44
5	22	37	22	30	28
6	60	75	62	58	55
7	30	45	32	35	28
8	44	71	42	40	46
9	26	42	25	28	21
10	100	131	117	96	104

Results are the mean of 5 assays set up on consecutive days
Buffer 1: PMN-Elastase, Merck Immunoassay, kit-buffer
Buffer 2: 0.15 mol/l NaCl, 0.025 mol/l phosphate with 1 g/l ovalbumin
Buffer 3: 0.15 mol/l NaCl, 0.025 mol/l phosphate, 0.15 ml/l Tween
Buffer 4: 0.15 mol/l NaCl, with 150 g/l bovine serum albumin
Buffer 5: 0.15 mol/l NaCl, 0.025 mol/l phosphate, 0.05 mol/l Tris-HCl 1 : 1 mixture containing 2.5 g/l bovine serum albumin, 0.15 ml/l Tween
All buffers had a pH of 7.6.

Tab. 4. Recovery studies on the elastase-α_1-proteinase inhibitor complex and lactoferrin concentrations in plasma samples.

1. *Elastase-α_1-proteinase inhibitor*

Mean recovery at 4 dilutions of one plasma added to a constant amount of a second plasma sample. Mean results from 4 assays, dilution of plasma 3 in assay buffer 3 (cf. tab. 3).

Plasma 3 µg/l	Plasma 4 µg/l	Expected µg/l	Found µg/l	Recovery %
1170 (1 : 1)	93	1263	1210	96
585 (1 : 2)	93	678	687	101
293 (1 : 4)	93	386	367	95
146 (1 : 8)	93	239	244	102

2. *Lactoferrin*

Mean recovery from an admixture of two plasma samples. Results from 5 assays, plasma 5 — 1.25 mg/l, plasma 2 — 0.12 mg/l.

Plasma 5 Plasma 6 mixture ratio	Expected mg/l	Found mg/l	Recovery %
1 + 0	1.25	1.28	102
1 + 1	0.68	0.65	96
1 + 2	0.49	0.47	96
1 + 4	0.35	0.36	103
1 + 8	0.24	0.22	92

The lactoferrin assay was specific for lactoferrin and did not cross-react with transferrin, even when the latter was present at a concentration of 10 g/l.

In the lactoferrin assay, the same results were obtained after 30, 60 and 90 minutes incubation times ($y = a + bx$) $n = 25$ different plasma samples in each case. For 30 min v 60 min $r = 0.988$; $a_{yx} = 0.002$; $b_{yx} = 0.981$. This validation data was needed to reduce the time between taking the sample and receiving the results.

Table 5a shows the reference range established for elastase-α_1-proteinase inhibitor complexes in EDTA plasma from healthy blood donors and the concentrations of this analyte in dialysis patient plasma.

Table 5b shows the same for lactoferrin values in EDTA plasma and in serum taken after the blood had been allowed to clot for at least 30 minutes. The difference between plasma and serum was statistically significant for lactoferrin (*Mann-Whitney* U-test: $z = 3.94$, $p < 0.01$).

The difference before and after dialysis was statistically significant for both elastase and lactoferrin (*Wilcoxon* signed rank test: c_α elastase $= 6.23$, c_α lactoferrin $= 5.90$, $p < 0.01$ in both cases).

Tab. 5. Concentrations of elastase-α_1-proteinase inhibitor complexes and lactoferrin in plasma from uraemic patients under dialysis treatment, in comparison with healthy blood donors.

5a. *Elastase*

Percentile	Reference population	Haemodialysis patients	
	plasma µg/l	before µg/l	after µg/l
2.5	68	112	258
16	88	140	367
50 (median)	113	205	636
84	144	365	1411
97.5	183	1132	2842
mean/median	1.03	1.45	1.27
n	51	37	37

5b. *Lactoferrin*

Percentile	Reference population		Haemodialysis patients	
	plasma mg/l	serum mg/l	before mg/l	after mg/l
2.5	<0.05	<0.06	<0.05	<0.05
16	0.11	0.17	0.18	0.36
50 (median)	0.17	0.32	0.27	0.56
84	0.31	0.68	0.46	1.06
97.5	0.42	1.66	1.12	2.25
mean/median	1.17	1.40	1.17	1.25
n	104	125	37	37

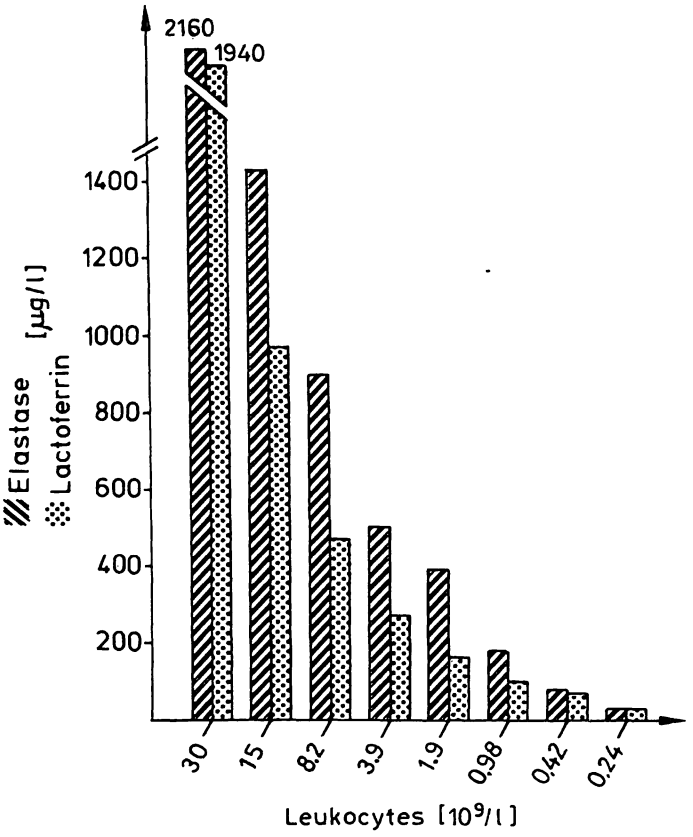

Fig. 2. Serial dilution in 0.15 mol/l NaCl of a leukocyte preparation. The concentration of elastase-α_1-proteinase inhibitor complexes and lactoferrin in the lysate (2x freeze-thaw-cycles) is shown on the ordinate (values for both analytes in µg/l). The numbers of leukocytes per liter is shown on the abscissa.

Figure 2 shows the correlation of elastase (measured in complex with added α_1-proteinase inhibitor) and lactoferrin in the supernate of a leukocyte preparation after serial dilution, centrifugation, and two freeze-thaw cycles. Before freezing and thawing, the supernate contained no measurable elastase or lactoferrin.

Figure 3 shows the values for elastase α_1-proteinase inhibitor and lactoferrin in bronchoalveolar lavage in selected patients with chronic obstructive lung disease and bacterial pneumonia. The median concentration of elastase was 223 µg/l (range 230–1320) and of lactoferrin 0.20 mg/l (range < 0.05–3.05 mg/l). Figure 4 shows the correlation between the commercial enzyme immunoassay and the luminescent immunoassay in plasma from healthy and diseased patients.

Discussion

Lactoferrin can be determined rapidly and usually correlates with elastase concentrations, when the latter is determined in a complex with α_1-proteinase inhibitor (see tab. 5 and fig. 2). The lactoferrin results can be returned to the ward within one hour, which is important in the treatment of neonatal sepsis cases.

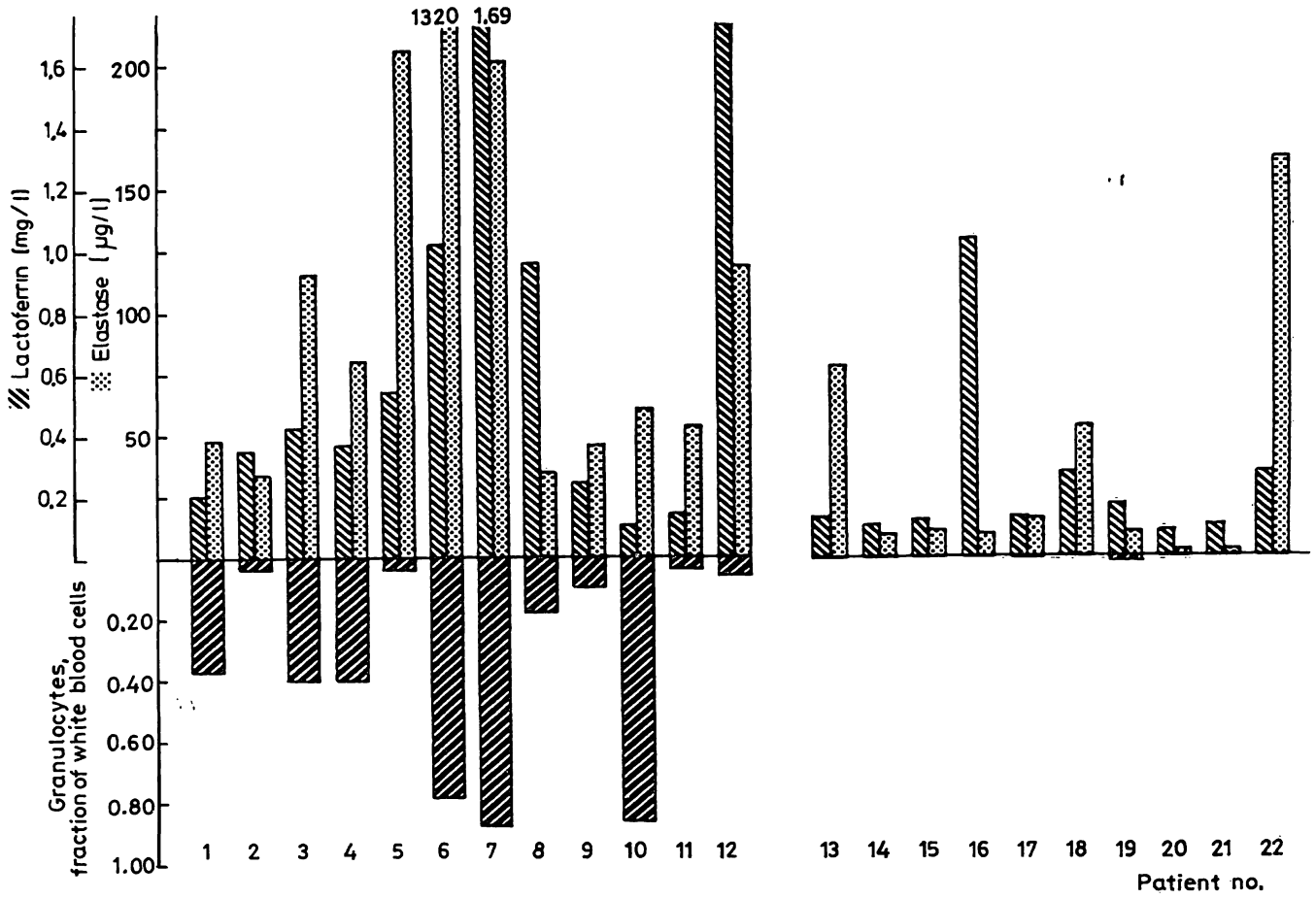

Fig. 3. Bronchoalveolar lavage from 12 patients with bacterial pneumonia (patients 1—12) and 10 patients with other chronic obstructive lung disease without bacterial involvement (patients 13—22). Concentrations of elastase-α_1-proteinase inhibitor complexes (expressed as µg/l elastase) and lactoferrin are shown in the ascending columns. The number of polymorphonuclear leukocytes, shown in the descending columns, is expressed as a fraction of the total cells found in the aspiration fluid. The data for the regression lines y = a + bx and x = a + by (elastase complex = x, lactoferrin = y) are: correlation coefficient r = 0.900,
ayx = 0.194, byx = 0.003
axy = −35, bxy = 318

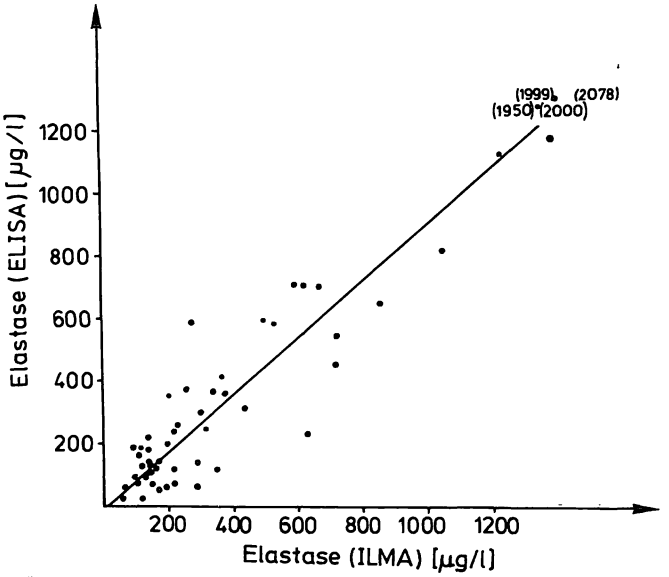

Fig. 4. Correlation between the Merck enzyme immunoassay (ELISA) and immunoluminometric assay (ILMA) for the determination of elastase-α_1-proteinase inhibitor complexes in body fluids. Data for 52 EDTA plasma samples taken from children aged 0—12 months. ELISA = 0.931 × ILMA − 43

It has been shown that lactoferrin is present in other body fluids and organs such as milk (7) and pancreas juice (6), so that plasma concentrations do not originate entirely from granulocyte destruction as in the case of granulocyte elastase. Although pancreatic elastase is also released in small amounts into the bloodstream, it does not react in this assay.

Both assays are simple to perform and are specific, sensitive and reproducible enough for determinations in plasma or lung lavage. The elastase ILMA is simpler to carry out than the elastase ELISA (see tab. 1a and 1b) because of the fewer wash and incubation steps.

The determination in bronchoalveolar lavage is theoretically more specific for lung sepsis cases because additional sources of elastase and lactoferrin are excluded, and because the concentrations are measured at the "site of action". Attempts at standardising the lactoferrin and elastase complex concentrations by relating them to albumin or urea in the bronchoal-

veolar lavage were abandoned as no improvement in separation between non-sepsis and sepsis groups was seen. This is in agreement with other findings (13, 15) for proteins in lavage.

The choice of assay buffer for the elastase-α_1-proteinase inhibitor ILMA was relatively unimportant, provided that ovalbumin was not added; addition of ovalbumin gave rise to higher results (tab. 3 column 2).

Leukocyte destruction during haemodialysis can be monitored using these assays (18, 19). Studies were performed before and after haemodialysis, to test the assays for expected increases in lactoferrin and elastase complexes (see tab. 5). The relationship of elastase-α_1-proteinase inhibitor complexes to lactoferrin was studied in these patients, as the literature mainly deals with elastase. The studies were performed as a prerun for testing leukocyte destruction using different haemodialysis membranes.

Unfortunately, commercial kits for elastase-α_1-proteinase inhibitor complexes are at present expensive and time consuming (see tab. 1a) although the producer has announced that a "rapid-version" of the assay will soon be available. The assay developed here correlated well with the commercial assay on plasma samples from infants, as figure 3 shows.

In measuring these two analytes, we monitor a paradox, inasmuch as the elastase released is capable of digesting lung tissue, when not complexed with α_1-proteinase inhibitor. On the other hand, lactoferrin has a bacteriostatic action (18–19) and promotes polymorphonuclear leukocyte adhesiveness (21).

The possibility cannot be excluded that lactoferrin also plays a role in removing active oxygen species such as free radicals and superoxide ions. It has been found that transferrin and ferritin are also elevated in bronchoalveolar lavage from such patients (23). All three proteins contain trivalent iron, which can be reduced to bivalent iron by active oxygen anions and radicals. In conclusion, assays are described for two components of polymorphonuclear leukocytes. Although the aim was to set up assays for monitoring cases of neonatal sepsis, it is possible to answer other questions regarding leukocyte activity and destruction by measuring these two compounds. Especially important is the fact that these assays provide reliable results of leukocyte involvement within an hour of receiving samples, which is crucial in neonatal intensive care.

References

1. Hansen, N. E., Malorquist, J. & Thorell, J. (1975) Acta Med. Scand. *198*, 437–443.
2. Neumann, S., Gunzer, G., Hennrich, N. & Lang, H. (1984) J. Clin. Chem. Clin. Biochem. *22*, 693–697.
3. Beatty, K., Bieth, J. & Travis, J. (1980) J. Biol. Chem. *255*, 3931–3934.
4. Duswald, K. H. (1980) Zur Pathobiochemie der Leukozyten-Elastase und ausgewählter Plasmaproteine bei Sepsis nach abdominal-chirurgischen Eingriffen. G-I-T Verlag, Darmstadt.
5. De Vet, B. J. C. M. & ten Hoopen, C. H. (1978) Acta Med. Scand. *203*, 197–203.
6. Colomb, E., Estevenon, J. P., Figarella, C., Guy, O. & Sarles, H. (1974) Biochim. Biophys. Acta *342*, 306–312.
7. Sørensen, M. & Sørensen, S. P. L. (1939) CR. Lab. Carlsberg, Ser. Clin. *23*, 55–99.
8. Plow, A. F. (1982) J. Clin. Invest. *69*, 564–572.
9. Bennet, R. M. & Mohla, C. (1976) J. Lab. Clin. Med. *88*, 156–166.
10. Wood, W. G. (1985) Luminescence Immunoassays in Theory and Practice – The State of the Art. In: Immunoassay Technology (Pal, S. B., ed.) Vol 1, De Gruyter, Berlin, New York, pp. 105–150.
11. Wood, W. G. (1984) J. Clin. Chem. Clin. Biochem. *22*, 905–918.
12. Wood, W. G., Braun, J. & Hantke, U. (1986) Meth. Enzymol. *133*, 354–365.
13. Braun, J., Schultek, T., Tegtmeier, K.-F., Florenz, A., Rohde, C. & Wood, W. G. (1986) Clin. Chem. *32*, 743–747.
14. Wood, W. G. & Gadow, A. (1983) J. Clin. Chem. Clin. Biochem. *21*, 789–797.
15. Schultek, T., Braun, J., Wießmann, K. J. & Wood, W. G. (1987) Dtsch. Med. Wochenschr. *112*, 1611–1614.
16. Wood, W. G., Waller, D. & Hantke, U. (1985) J. Clin. Chem. Clin. Biochem. *23*, 463–471.
17. Neumeier, D., Fateh-Moghadam, A. & Menzel, G. (1982) Fresenius' Z. Anal. Chem. *311*, 389–390.
18. Heidland, A., Hoerl, W., Heller, N., Heine, H., Neumann, S. & Heidbreder, E. (1983) Kidney International *24*, Suppl. 16, 27–36.
19. Hoerl, W., Steinhauer, H. B. & Schollmeyer, P. (1985) Kidney International *28*, 791–796.
20. Arnold, R. R., Cole, M. F. & Mc Ghee, J. R. (1977) Science *197*, 263–265.
21. Bullen, J. J. & Armstrong, J. A. (1979) Immunology *36*, 781–791.
22. Oseas, R., Yang, H. H., Baehner, R. L. & Boxes, L. A. (1981) Blood *57*, 939–945.
23. Boger, C. & Wood, W. G., Unpublished results.

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